

FIG._1A

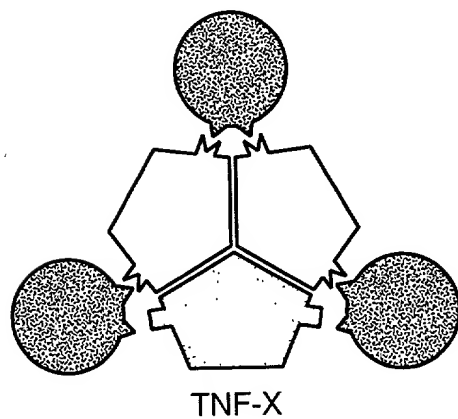


FIG._1B

200610-000000



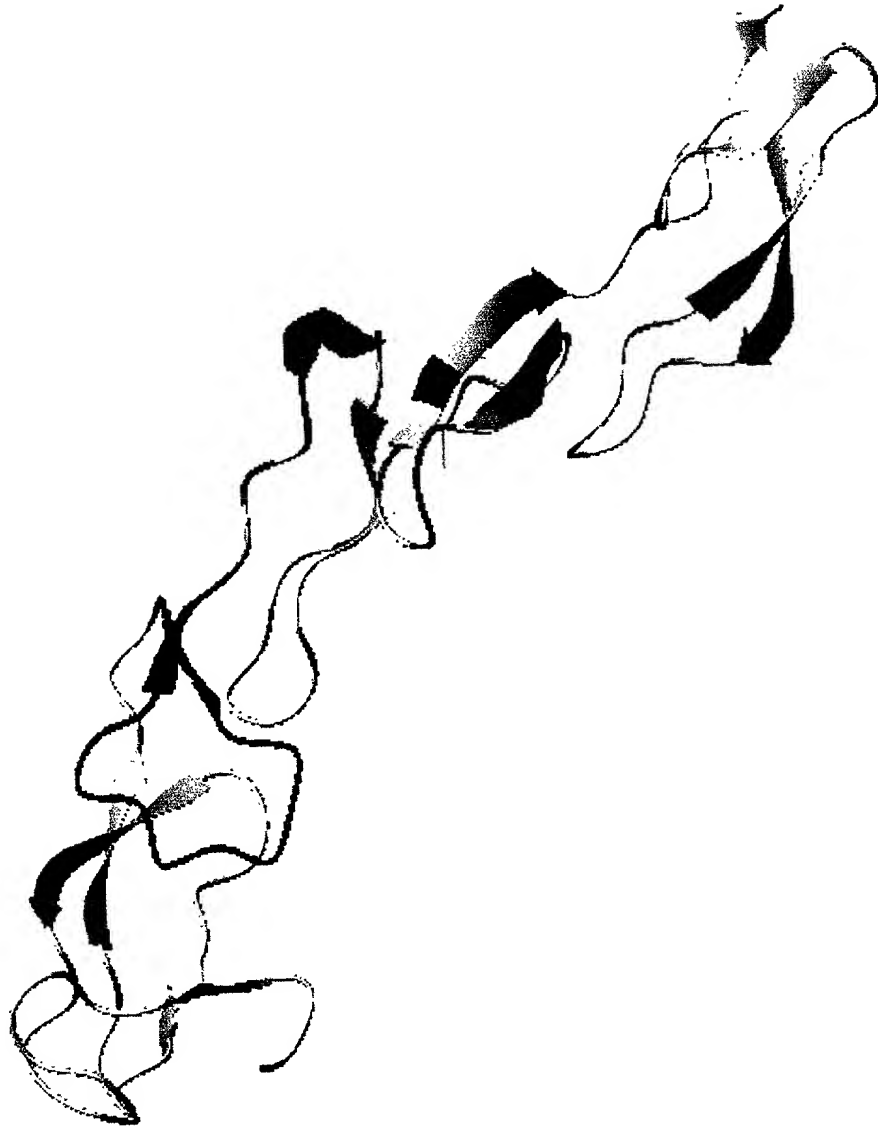
TNF-TNFR TRIMER COMPLEX

SIDE VIEW

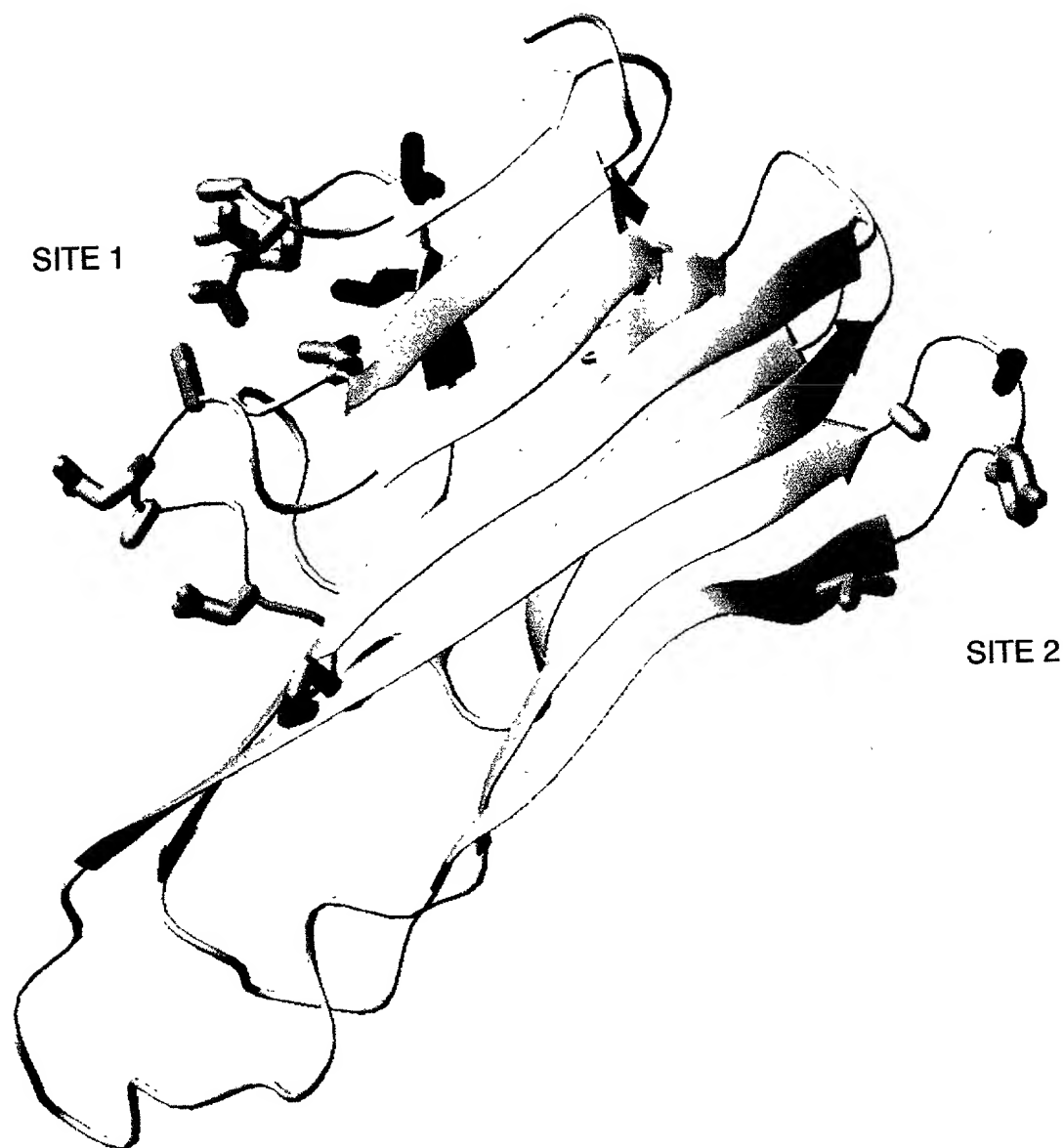
TOP VIEW

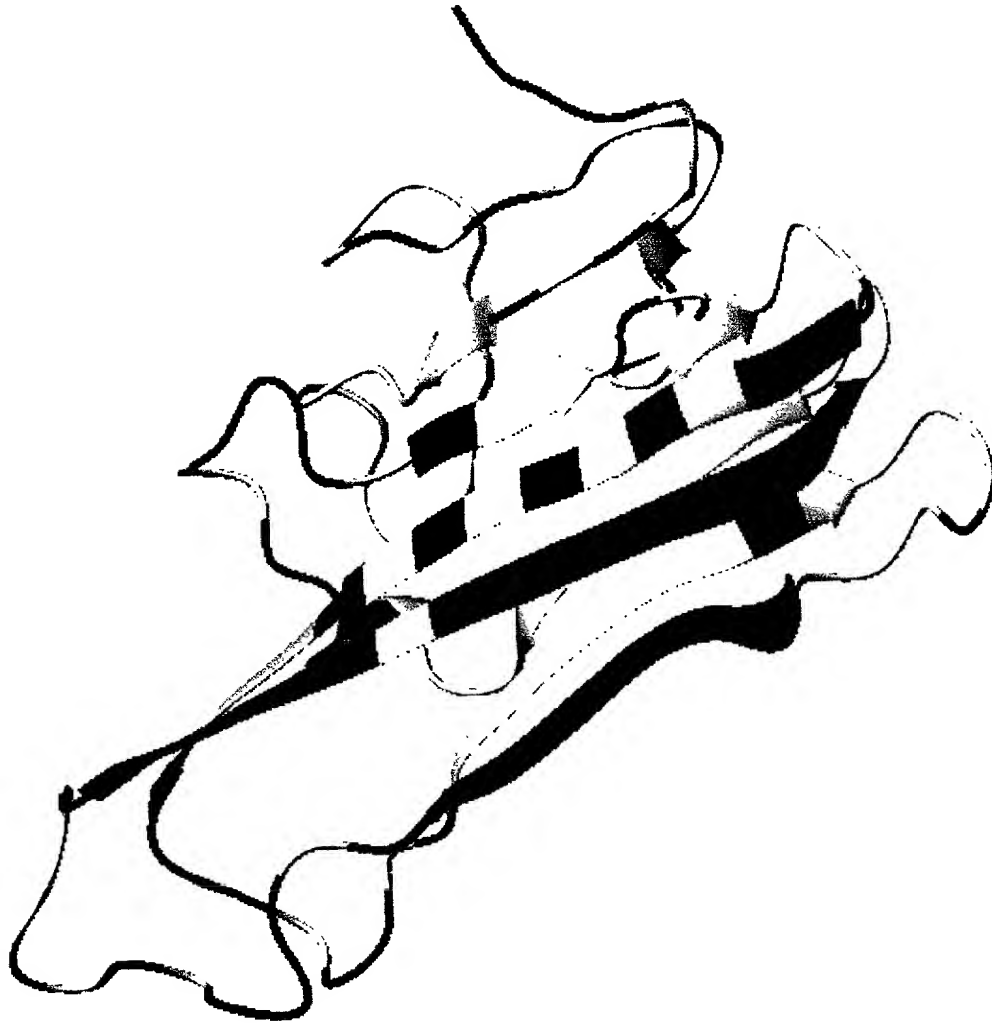


FIG.-2

**FIG._3**

PDB: 1B20

TNF α BINDING SITES**FIG._4**

TNF α TRIMER INTERFACE**FIG._5**

1 atgcaccacc accaccacca cgtacgctcc tectcccgea ctccgtecga caaacccggt
 61 gctcacgtag tagctaacc gcaggctgaa ggtcagctgc agtggtgaa ccgcccgcgt
 121 aacgctctgc tggctaaccg ttagaactg cgcgacaacc agctggtagt accgtccgaa
 181 ggtctgtacc tgatctactc ccagggtactg ttcaaaggtc agggttgtcc gtccactcac
 241 gtactgtgta ctacactat ctcccgcatc gctgtatcct accagactaa agtaaacctg
 301 ctgtccgcta tcaaateccc gtgtcagcgc gaaactccgg aagggtgctga agctaaaccg
 361 tggtagaac cgatctacct ggggtgtgta ttccagctgg aaaaagggtga ccgcctgtcc
 421 gctgaaatca accgcccggga ctacctggac ttcgctgaat ccggtcaggt atacttcggt
 481 atcatcgctc tgtga

FIG._6A

1 MHHHHHHVRS SSRTPSDKPV AHVVANPQAE GQLQWLNRRRA NALLANGVEL RDNQLVVPSE
 61 GLYLIYSQVL FKGQGCPSTH VLLTHTISRI AVSYQTKVNL LSAIKSPCQR ETPEGAEAKP
 121 WYEPIYLGCV FQLEKGDRLS AEINRPDYLD FAESGQVYFG IIAL

FIG._6B

Wild-type TNF amino acid	Wild-type TNF amino acid number	Mutants created
Q	21	R
N	30	D
R	31	I, D, E
R	32	D, E, S
A	33	E
A	35	S
K	65	D, T, M, W, I, Q, S, N, V, E
G	66	Q, K
Q	67	D, W, Y, R, K, S
A	111	R, E
K	112	D, E
Y	115	Q, K, E, N, R, F, H, M, L, I, W, D, T, S
D	140	R, K
D	143	E, N, Q, S, R, K
F	144	N
A	145	R, D, K, N, H, T, Q, E, Y, M, S, F
E	146	N, K, R, S
S	147	R

ALSO MADE DOUBLE MUTANTS K65E/D143K, K65E/D143R, K65D/D143K AND K65D/D143R

FIG._7

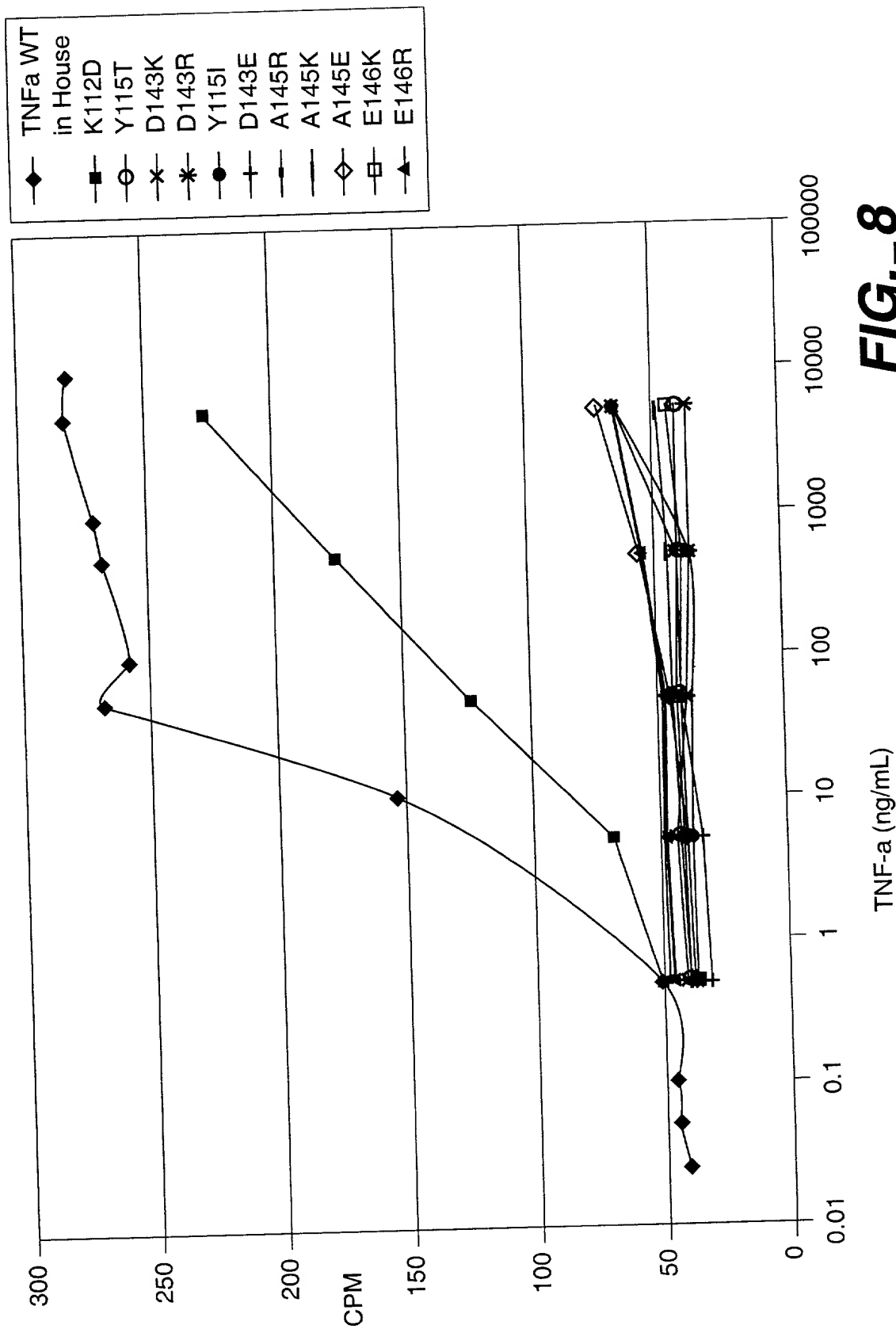


FIG._8

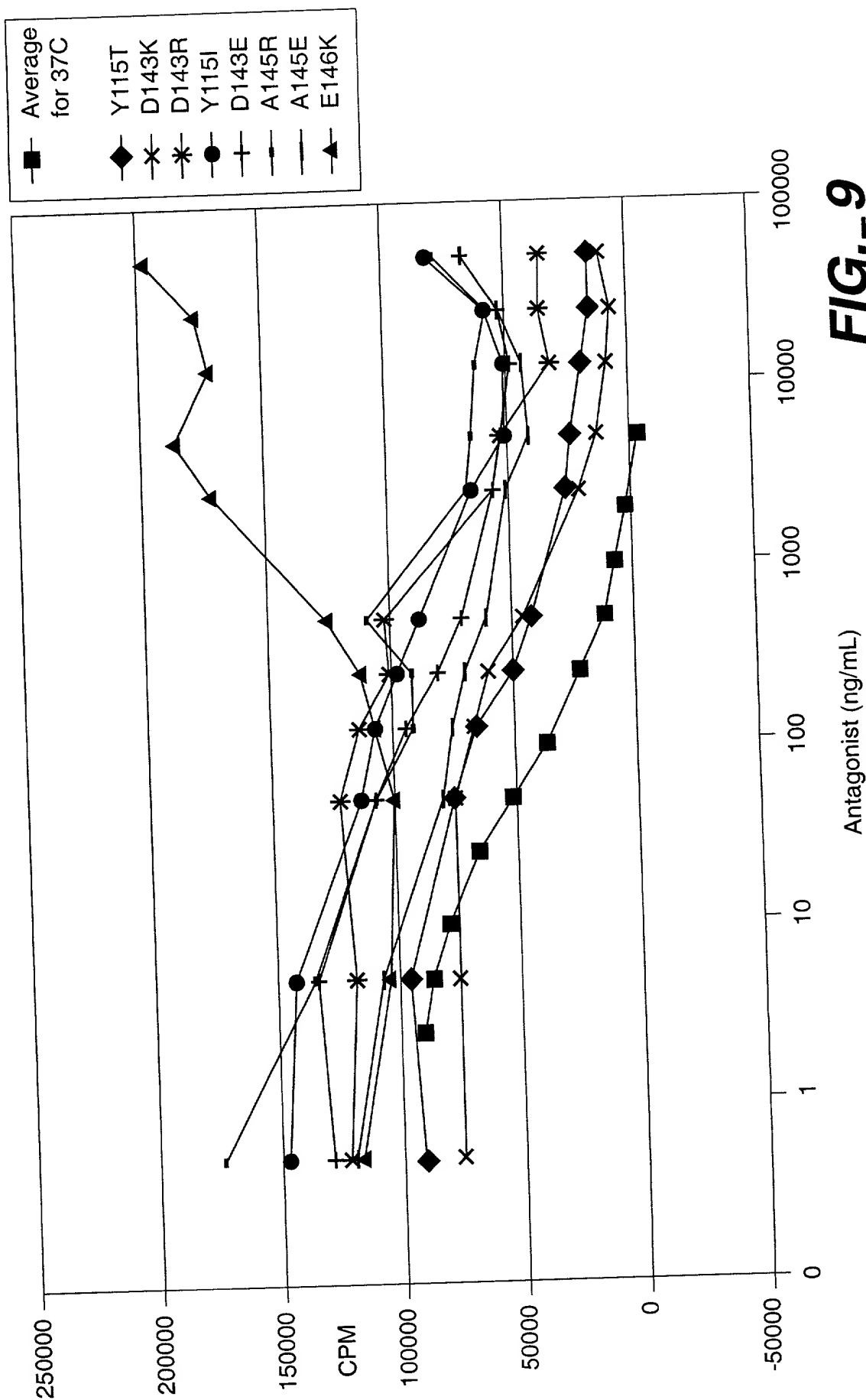


FIG. 9

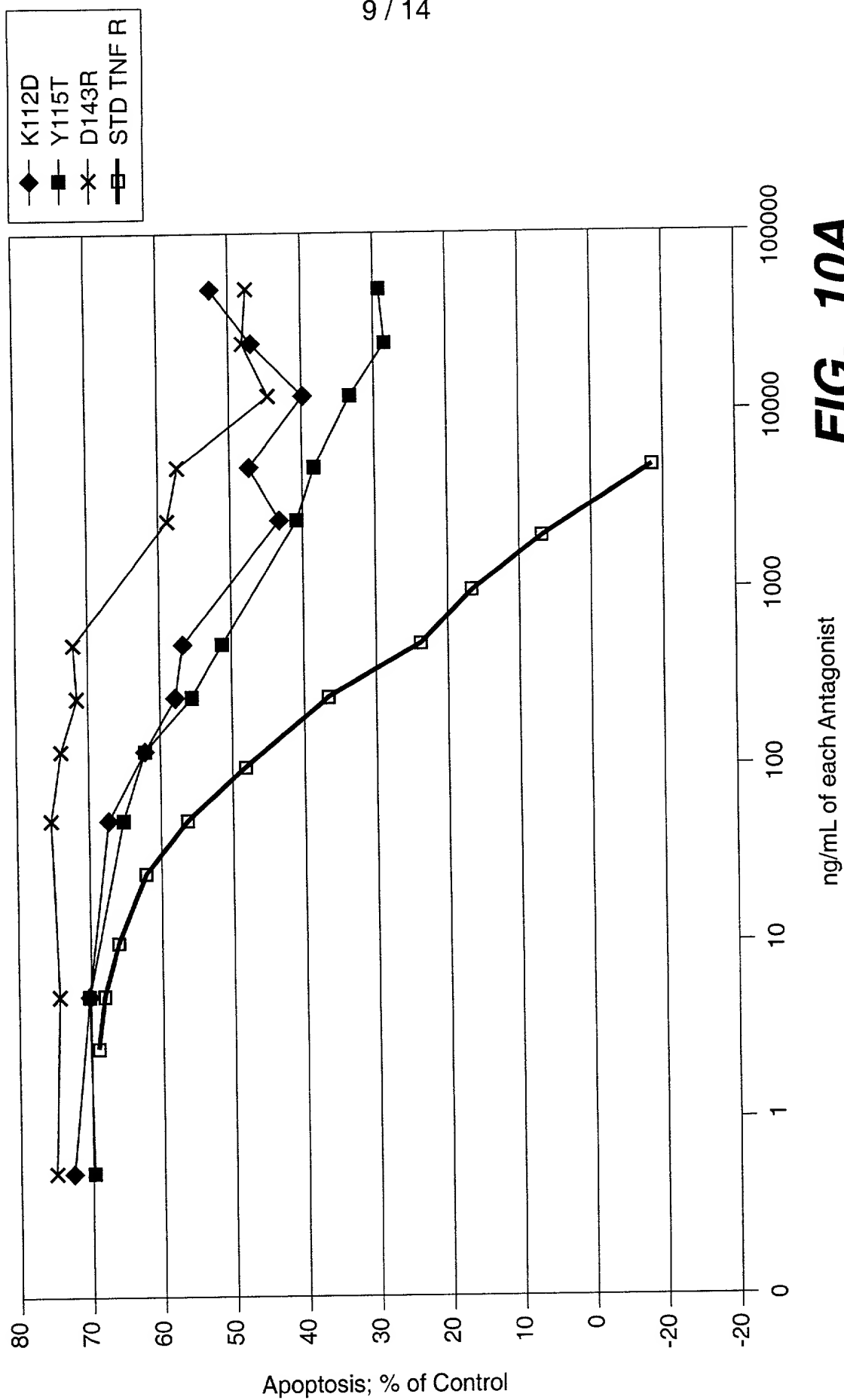
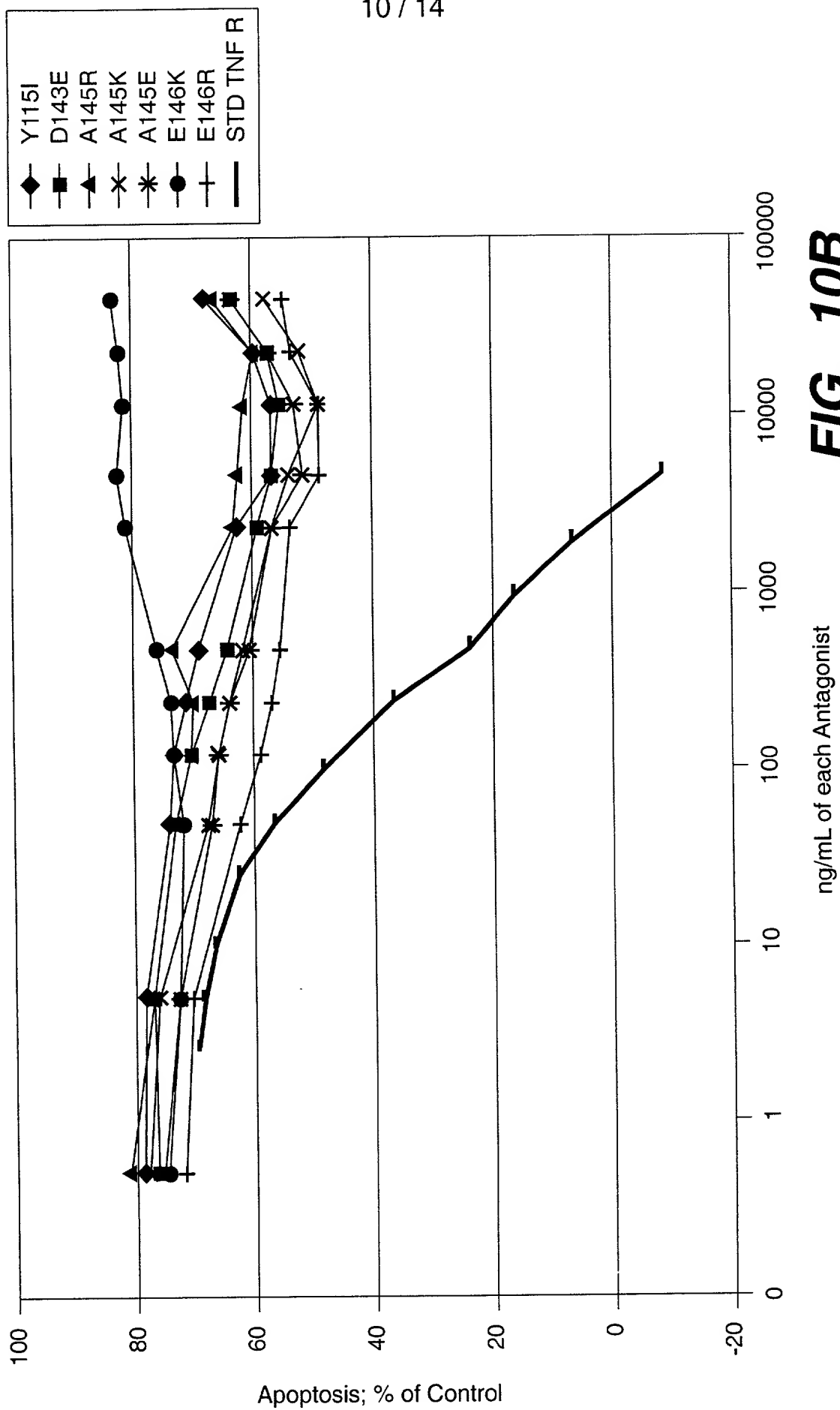


FIG. 10A



WT	PDA Relative Probability Distribution															
Q21	R1000															
N30	D1000															
R31	I1000															
R32	H1000															
A33	E1000															
A35	S1000															
K65	R585	D146	K110	T42	H31	M27	W15	I15	Q10	S9	N9	V1				
G66	Q813	K187														
Q67	D623	W209	Y83	R43	K41	S1										
A111	R959	E41														
K112	K1000															
Y115	Q230	K154	E116	N84	Y81	R72	F69	H43	M39	L36	I26	W25	D11	T8	S6	
D140	D1000															
L143	D680	E130	N110	Q33	S29	R12	K6									
F144	F695	N305														
A145	R456	D196	K124	N76	H67	T43	Q25	E9	Y1	M1	S1	F1				
E146	N489	K377	R111	D12	S10	E1										
S147	R1000															

FIG._ 11

TRAF2(310-) DQDKIEALSSKVQQQLERSIGLKDLAMADLEQKVLEMEA STYDG

FIG._12A

TRAF3(374-) VARNTGLLESQLSRHDQMLSVHDIRLADMDLRFQVLET ASYNG

FIG._12B

TRAF5(343-) NDQRLAVLEEETNKHDTHINIHKAQLSKNEERFKLLEG TCYNG

FIG._12C

TRAF1(225-) DRERILSLEQRVVVELQQTALAQKDQALGKLEQSLRLMEE ASFDG

FIG._12D

TRAF6(309-) QDHQIRELTAKMETQSMYVSELKRTIRTLEDKVAEIEA QQCNG

FIG._12E

TRAF4(201-) -----CALVSRQRQELQELRRELEELSV GS-DG

FIG._12F

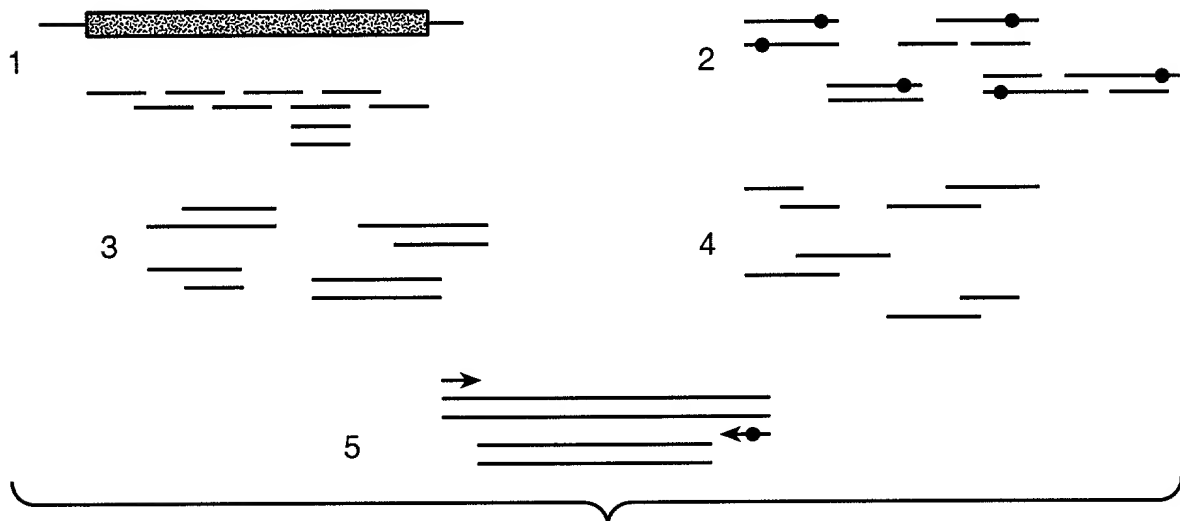


FIG._13

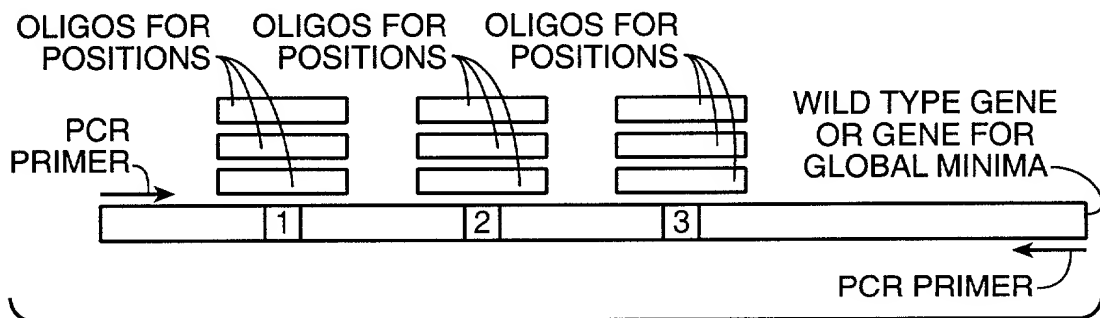
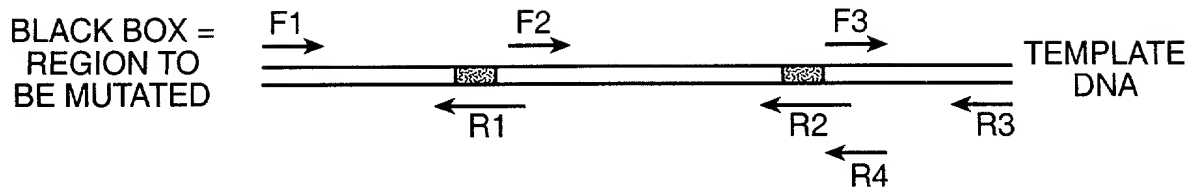


FIG._14



STEP 1: SET UP 3 PCR REACTIONS:

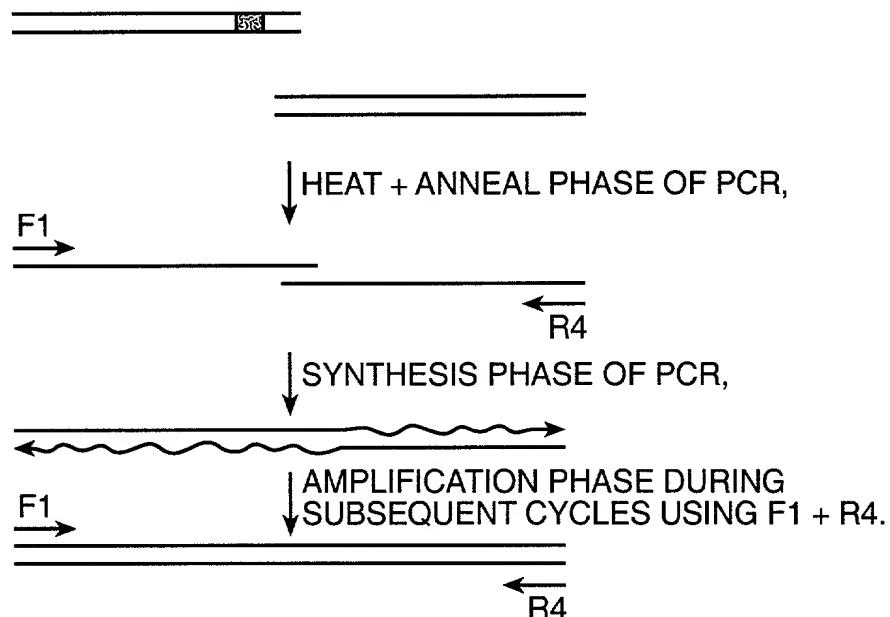
PRODUCTS:

TUBE 1:

TUBE 2:

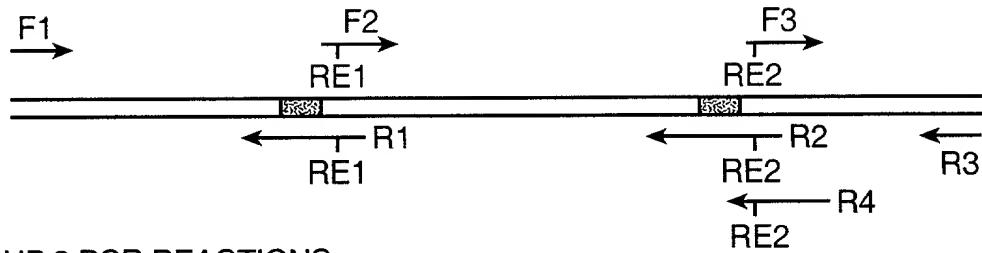
TUBE 3:

STEP 2: SET UP PCR REACTION WITH PRODUCTS OF TUBE 1 + PRODUCTS TUBE 2 + F1 + R4.



STEP 3: REPEAT STEP 2 USING PRODUCT FROM STEP 2 + PRODUCT FROM STEP 1, TUBE 3 + PRIMERS F1 + R3.

FIG. 15



STEP 1: SET UP 3 PCR REACTIONS:

TUBE 1:
 A horizontal DNA template with a shaded rectangular restriction site labeled RE1. Below the template, primer R1 is indicated with an arrow pointing left.

TUBE 2:
 A horizontal DNA template with two shaded rectangular restriction sites labeled RE1 and RE2. Below the template, primers R1 and R2 are indicated with arrows pointing left. R1 is between RE1 and RE2, and R2 is between RE2 and the right end.

TUBE 3:
 A horizontal DNA template with a shaded rectangular restriction site labeled RE2. Below the template, primer R2 is indicated with an arrow pointing left.

STEP 2: DIGEST PRODUCTS FROM STEP 1 WITH SUITABLE RESTRICTION ENDONUCLEASES.

STEP 3: LIGATE DIGESTED PRODUCT FROM STEP 2, TUBE 2 WITH DIGESTED PRODUCT FROM STEP 2, TUBE 1.



STEP 4: AMPLIFY VIA PCR LIGATED PRODUCTS OF STEP 3 WITH F1 + R4.



STEP 5: DIGEST AMPLIFIED PRODUCT OF STEP 4 WITH RESTRICTION ENDONUCLEASE #2.



STEP 6: LIGATE PRODUCT FROM STEP 5 WITH PRODUCT FROM STEP 2, TUBE 1.



STEP 7: AMPLIFY PRODUCT FROM STEP 6 WITH F1 + R3.

FIG._16

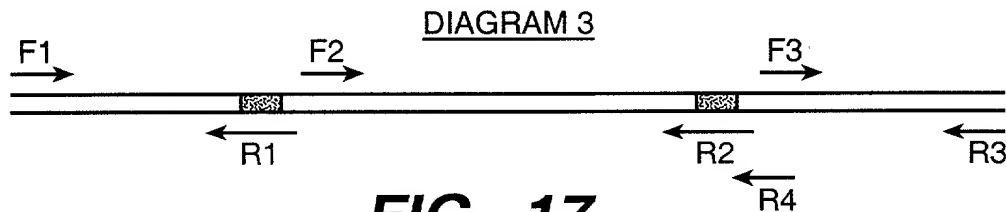


FIG._17